# RNA INTERFERENCE IN AGRICULTURE: METHODS, APPLICATIONS, AND GOVERNANCE


Chairs

**Ana M. Vélez Arango**

Associate Professor, Department of Entomology, University of Nebraska-Lincoln, Lincoln, NE

**Kenneth Narva**

Head of Entomology, GreenLight Biosciences, Inc., Durham, NC



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The Science Source for Food, Agricultural, and Environmental Issues

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- Nonpartisan and apolitical
- Membership includes 27 scientific societies; 20 universities; 19 libraries; 45 nonprofits; 21 companies; and over 500 individuals from 46 states and 7 countries
- Celebrated its 50th anniversary in 2022

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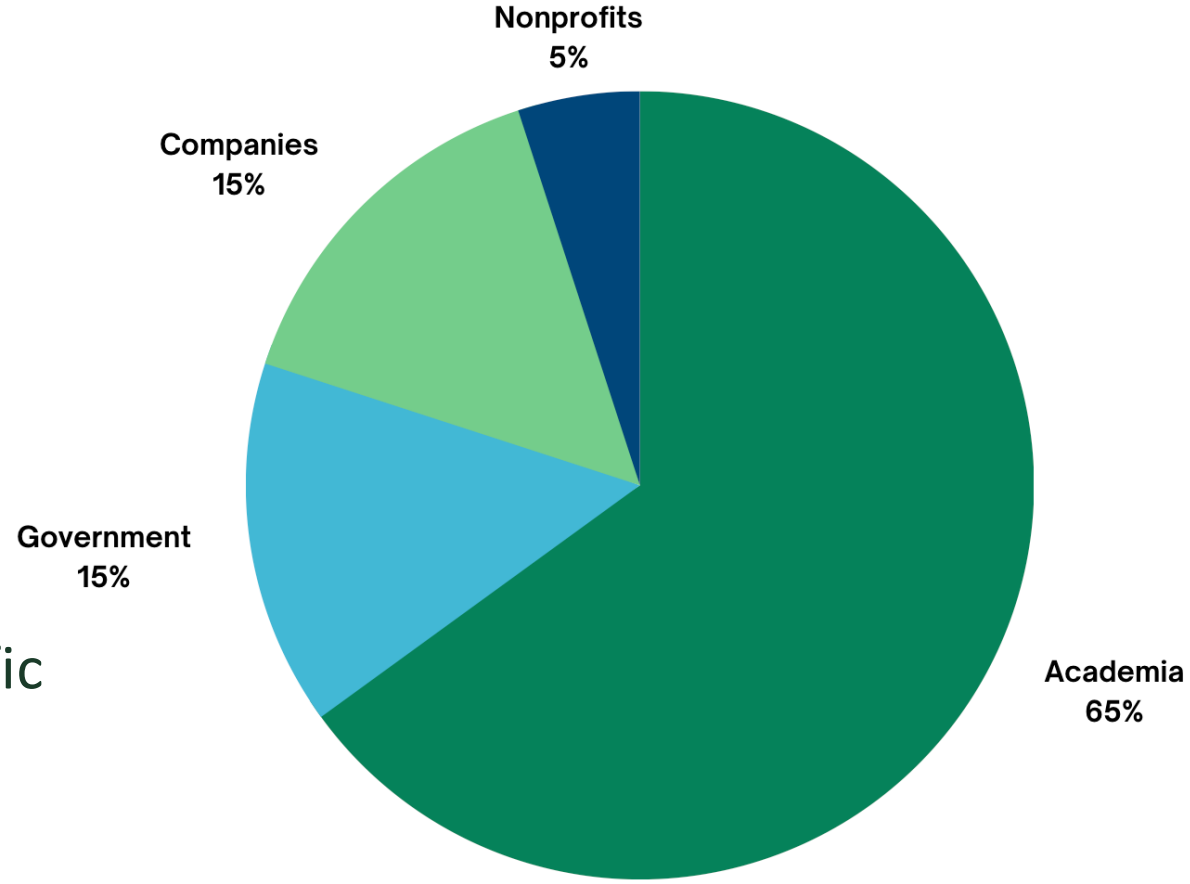
- Nearly 200 active task force members working on CAST reports yet to be released
- Volunteer scientific experts as authors and reviewers—more than 1800 volunteers since 2008



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## Authors


**Molly Darlington**
Postdoctoral Research Associate
University of Nebraska-Lincoln
Lincoln, NE

**Juan Luis Jurat-Fuentes**
Professor
University of Tennessee
Knoxville, TN

**Karl-Heinz Kogel**
Professor
Justus-Liebig-Universität Gießen
Gießen, Hesse, Germany

**Keerti S. Rathore**
Professor
Texas A&M University
College Station, TX

**Guy Smagghe**
Professor
Department of Plants and Crops, Ghent University
Ghent, Belgium

**Steve Whyard**
Professor
University of Manitoba
Winnipeg, Manitoba


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Head of State Regulatory Affairs
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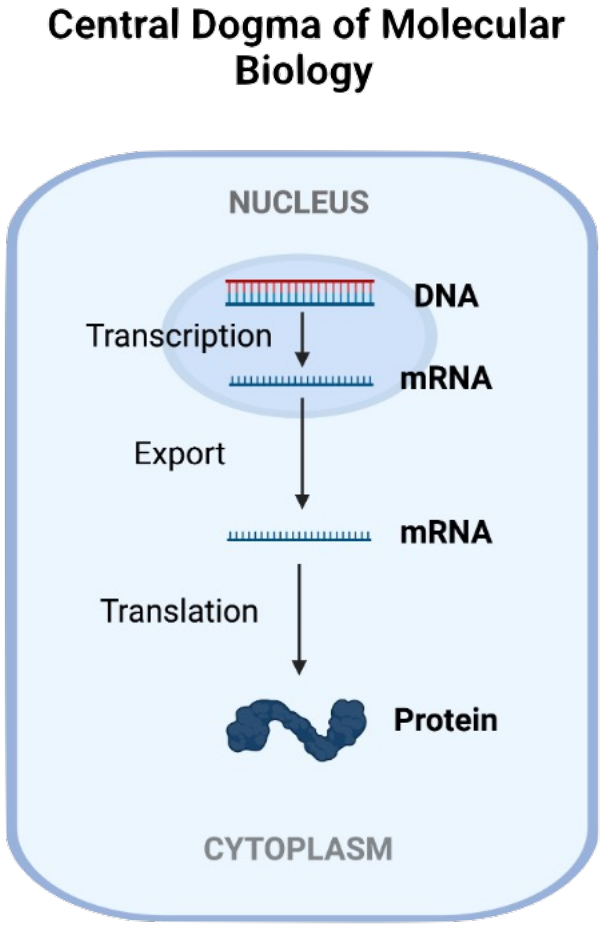
**Blair Siegfried**
Associate Dean for Research and Graduate Education
Penn State University
University Park, PA

# Outline

- What is RNAi?
- Role of RNAi in Agriculture
- Applications
- Regulatory Considerations
- Products
- Challenges
- Future Innovations

# What is RNAi?


Central Dogma of Molecular Biology
NUCLEUS
DNA
Transcription
mRNA
Export
mRNA
Translation
Protein
CYTOPLASM

# What is RNAi?

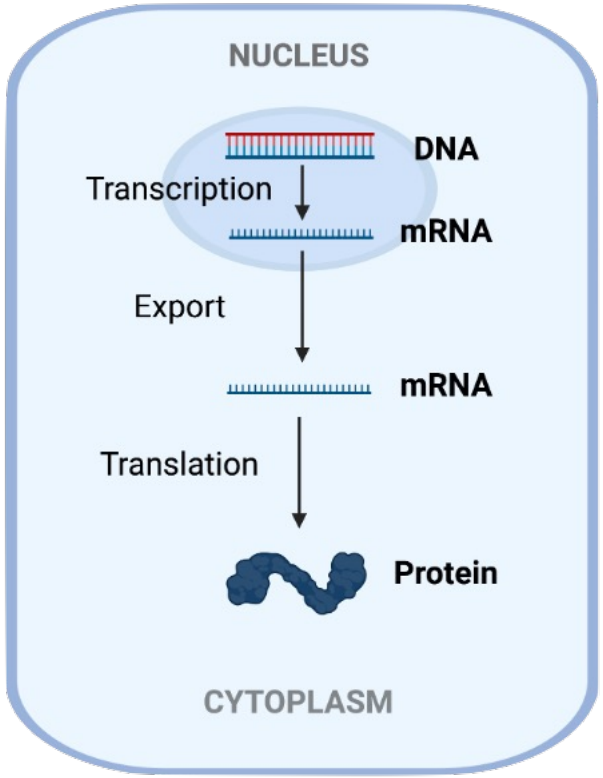
## RNA Interference Discovery

Petunias transformed to over-express the enzyme associated with purple pigmentation

mRNA coding for purple protein

Credits (Richard Jorgensen): University of Arizona

**Central Dogma of Molecular Biology**



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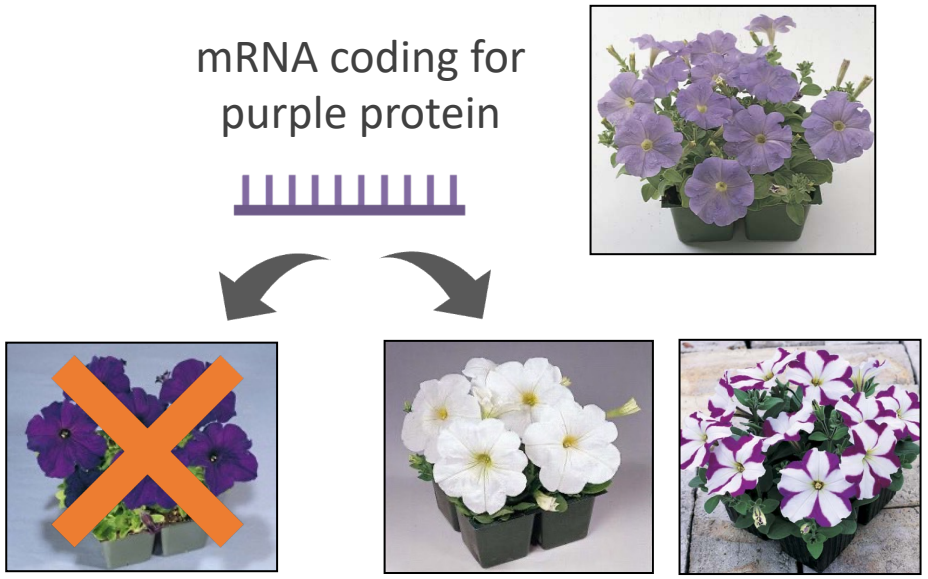


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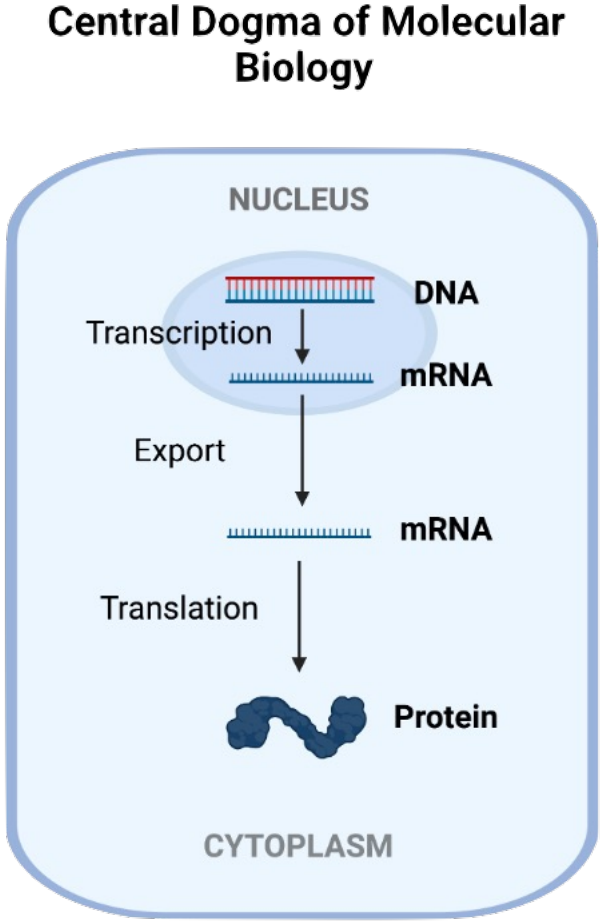

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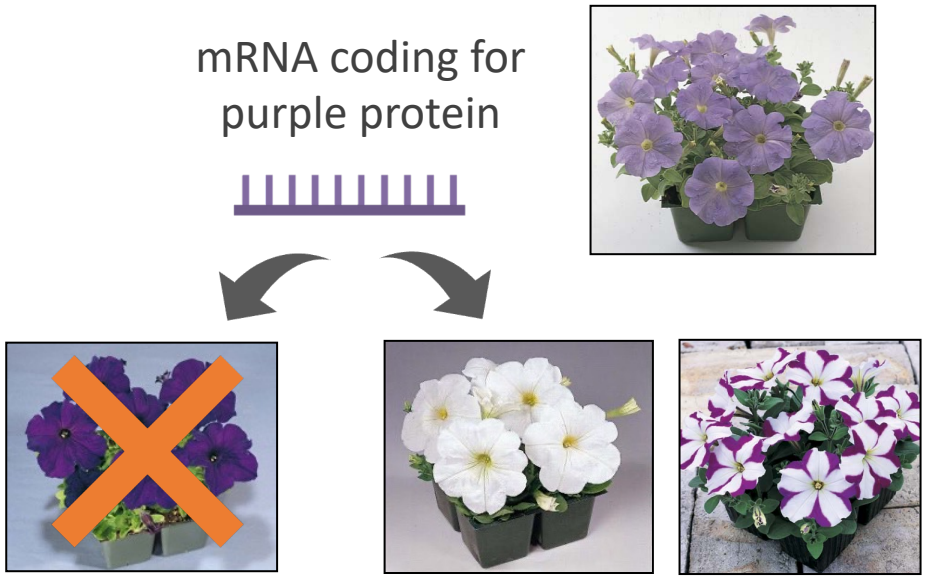


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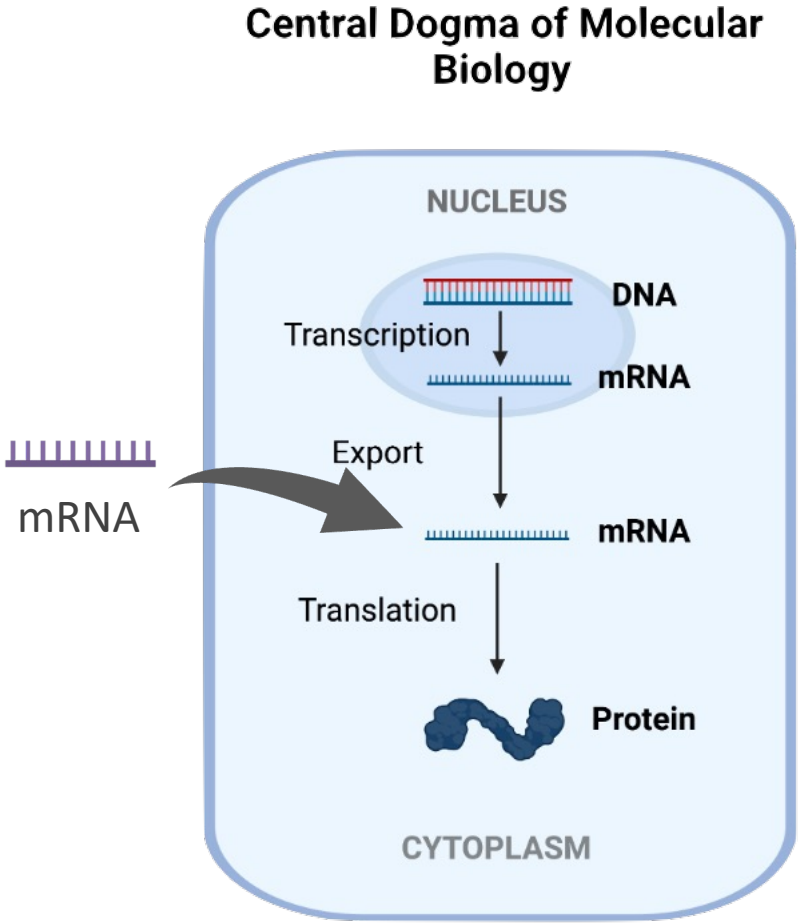

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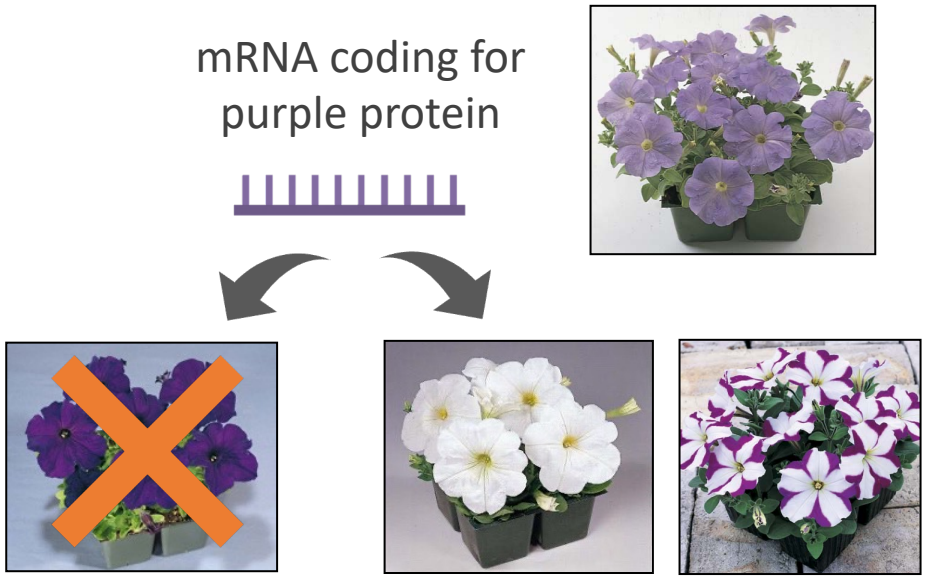


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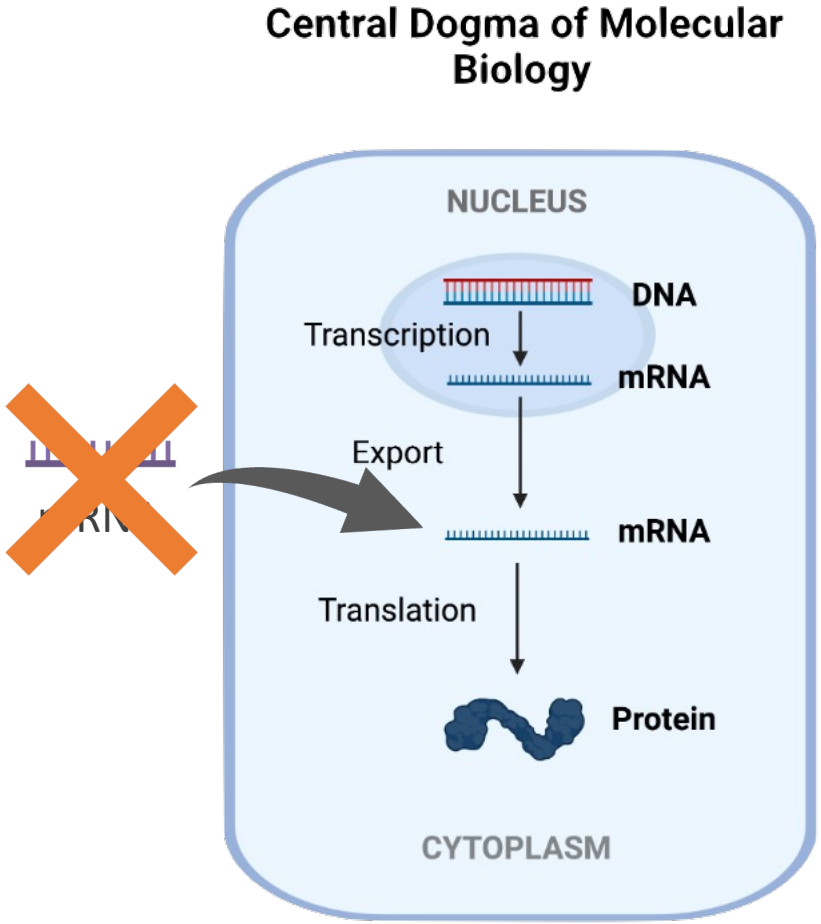
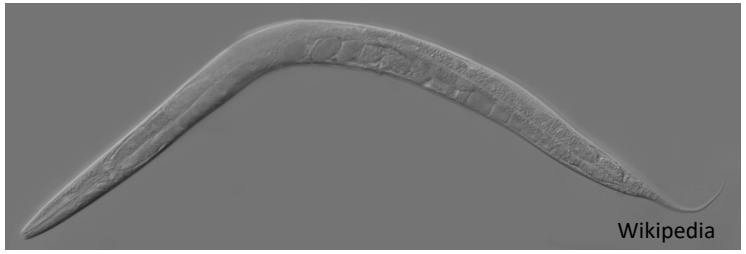


# What is RNAi?

## RNA Interference Discovery

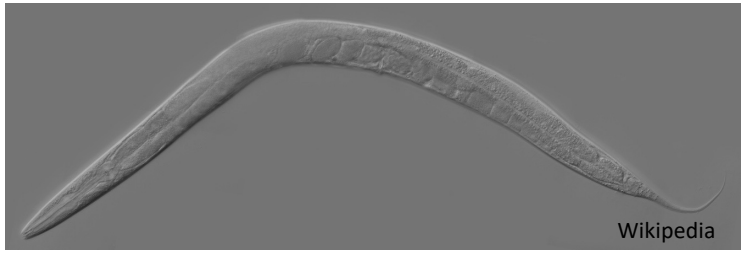



Roundworm, *Caenorhabditis elegans*

Andrew Fire
Craig Melo
Nobel Prize in Physiology and Medicine in 2006

# What is RNAi?

## RNA Interference Discovery

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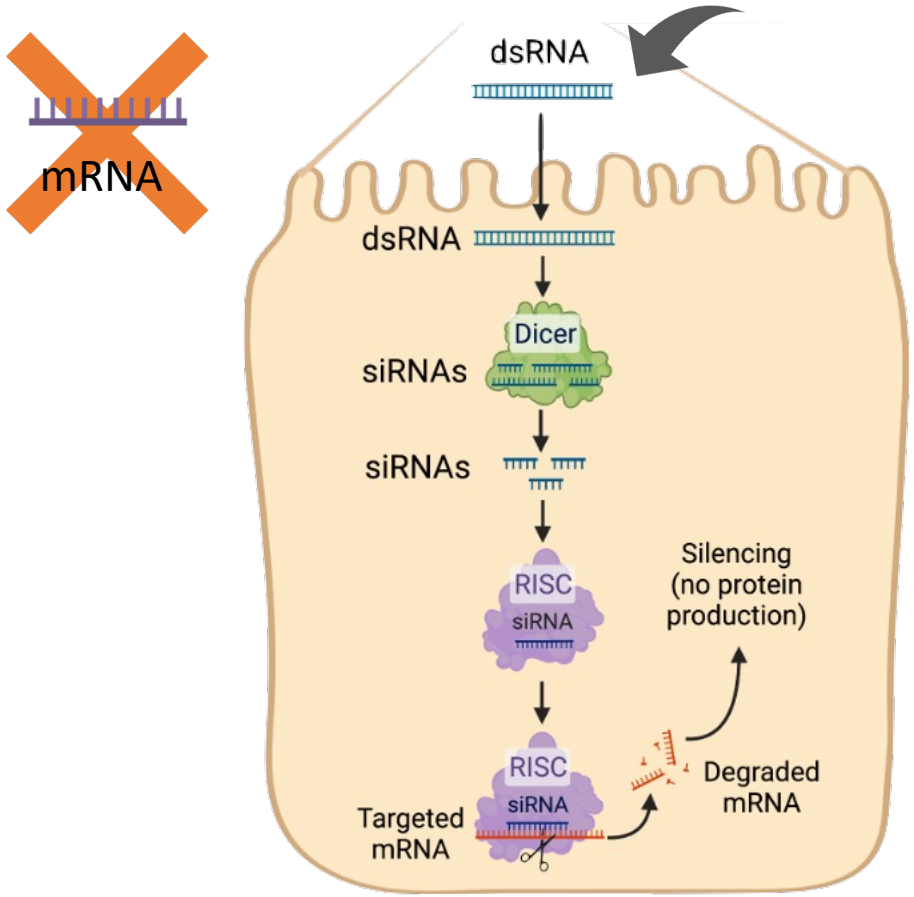
Post-transcriptional gene silencing mechanism



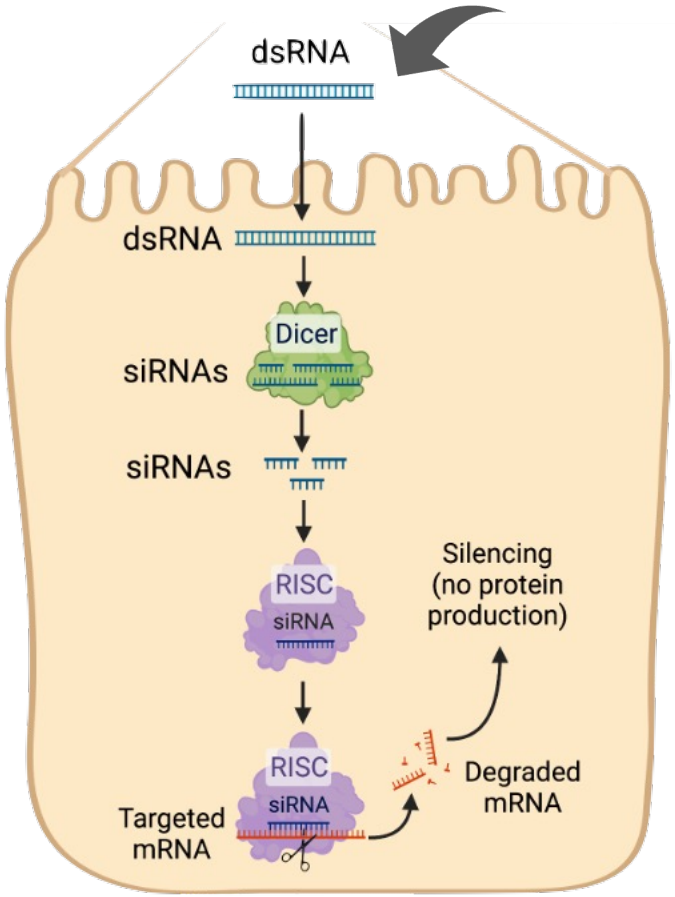
# What is RNAi?

## RNA Interference Discovery

- dsRNA leads mRNA degradation that prevents protein formation → **gene silencing**

- Innate mechanism conserved in eukaryotes
  - Gene regulation
  - Defense against virus

Post-transcriptional gene silencing mechanism

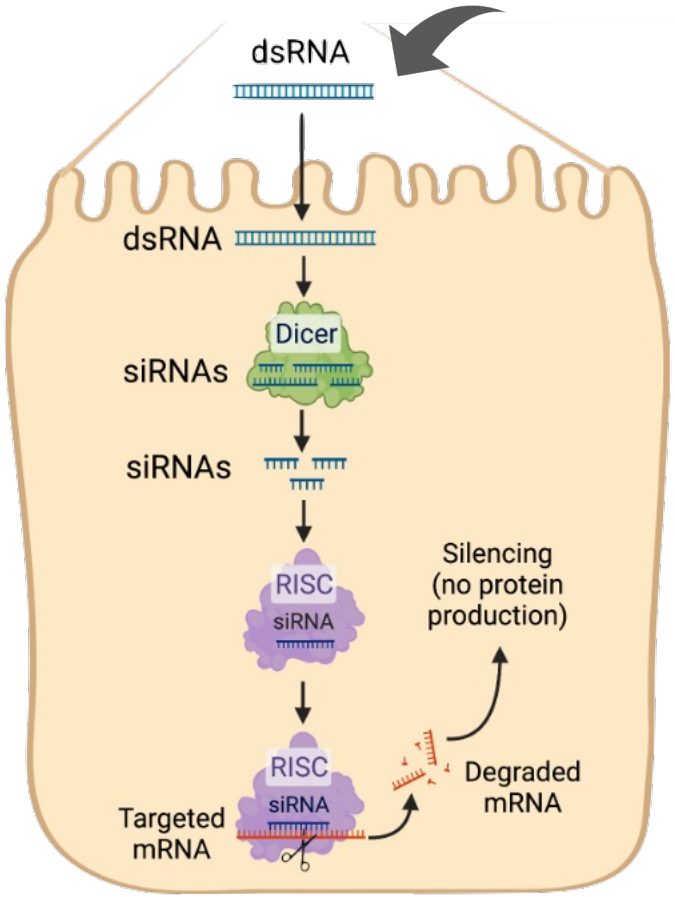


# What is RNAi?

## Concepts

- **RNAi** → mechanism
- **dsRNA** → molecule entering the cell
- **siRNA** → active molecule
- **Gene silencing or knockdown** → result (no protein production)

Post-transcriptional gene silencing mechanism



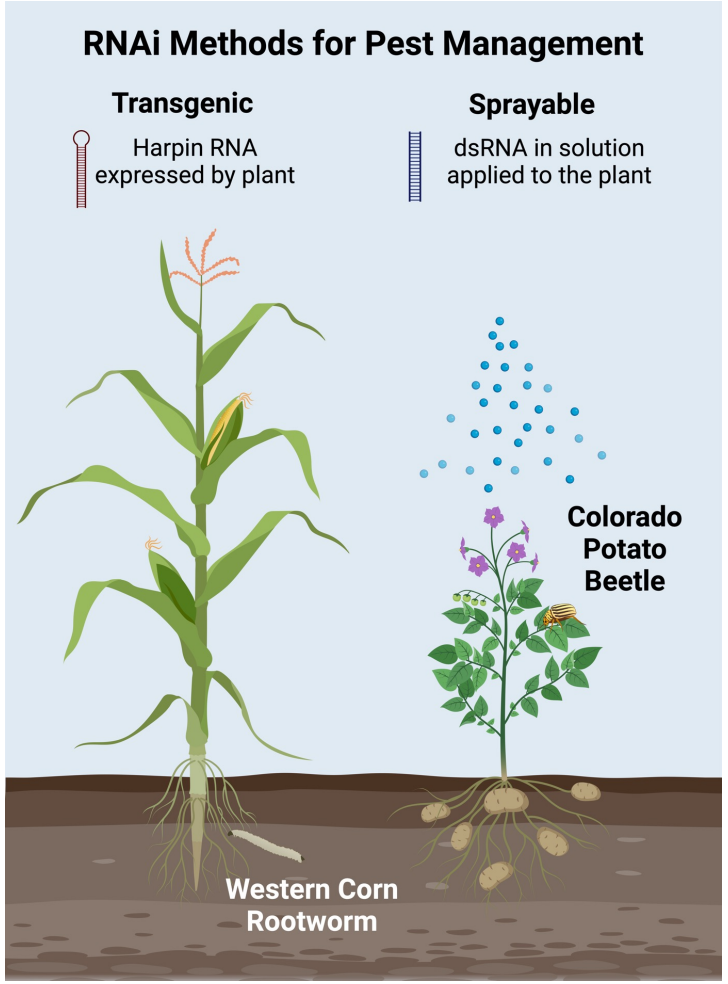
# Role of RNAi in Agriculture

## Crop protection against insect pests and pathogens

Knockdown genes important for the organism to function with dsRNA

↓

Lethal or sublethal effects



# Role of RNAi in Agriculture

## Crop protection against insect pests and pathogens

### Advantages

- New Mode of Action (MoA)
  - IRAC Group 35 sprayable insecticide
- Reduce reliance on synthetic chemistries
- Highly specific biopesticide
- Low environmental persistence
- Low residue on crops

# Role of RNAi in Agriculture

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- New Mode of Action (MoA)
  - IRAC Group 35 sprayable insecticide
- Reduce reliance on synthetic chemistries
- Highly specific biopesticide
- Low environmental persistence
- Low residue on crops

### Disadvantages

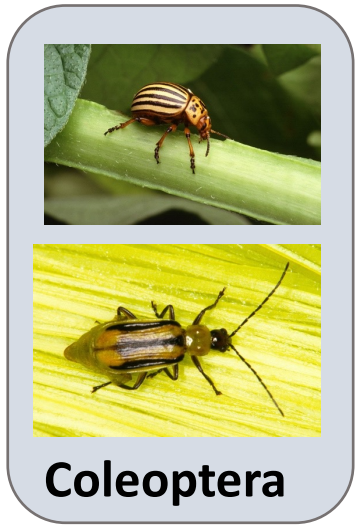
- Variability in response between organisms
- Slower acting compared to synthetic chemistries
- Narrow activity spectrum

# Role of RNAi in Agriculture

## Crop protection against insect pests and pathogens

Variability in the response between insects

- Orders
- Species
- Life stages
- Tissues
- Genes

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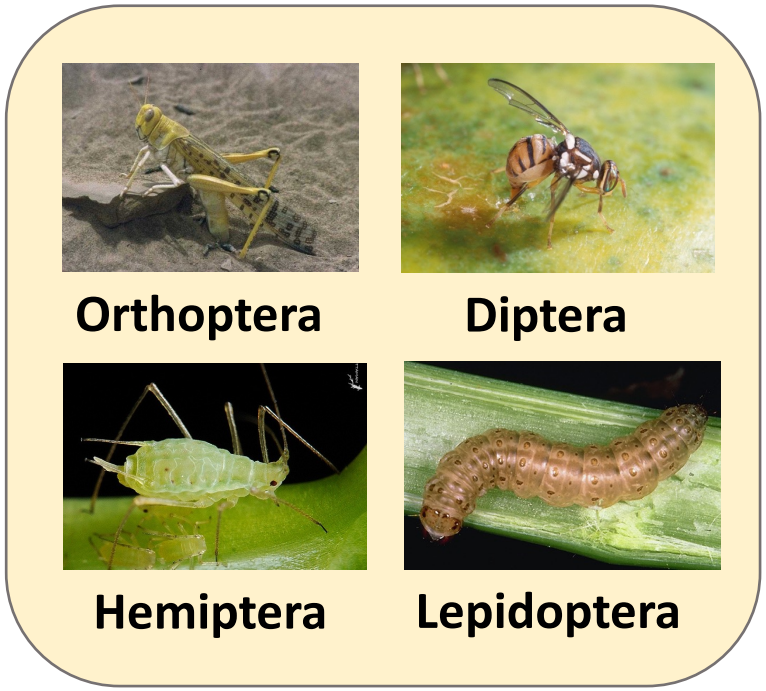



Christiaens *et al*. 2020. *Frontiers in Plant Sciences*, 11: 451

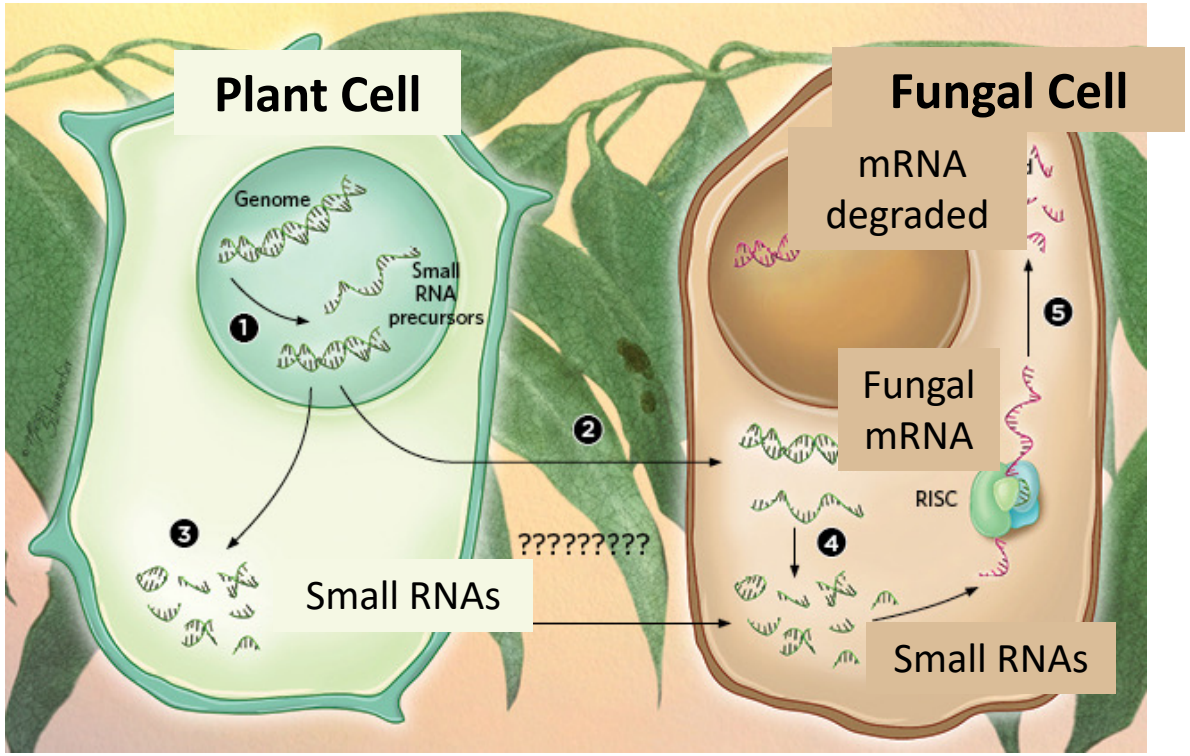
# Role of RNAi in Agriculture

## Crop protection against insect pests and pathogens

Variability in the response between fungi

- Cross-kingdom RNAi
  - Unclear if dsRNA uptake is common

Weiberg *et al*. 2013. *Science*, 342:118–123.




Grens. 2017. The Scientists.

# Applications: Genetically Modified Crop

## GM corn roots protected by dsRNA targeting corn rootworm

(-) dsRNA


(+) dsRNA

Baum *et al.* 2007. *Nat Biotechnol.,* 25:1322–1326.

# Applications: Sprayable dsRNA Biopesticide

## Potato plants protected by dsRNA targeting Colorado potato beetle

Untreated

Image courtesy of GreenLight Biosciences

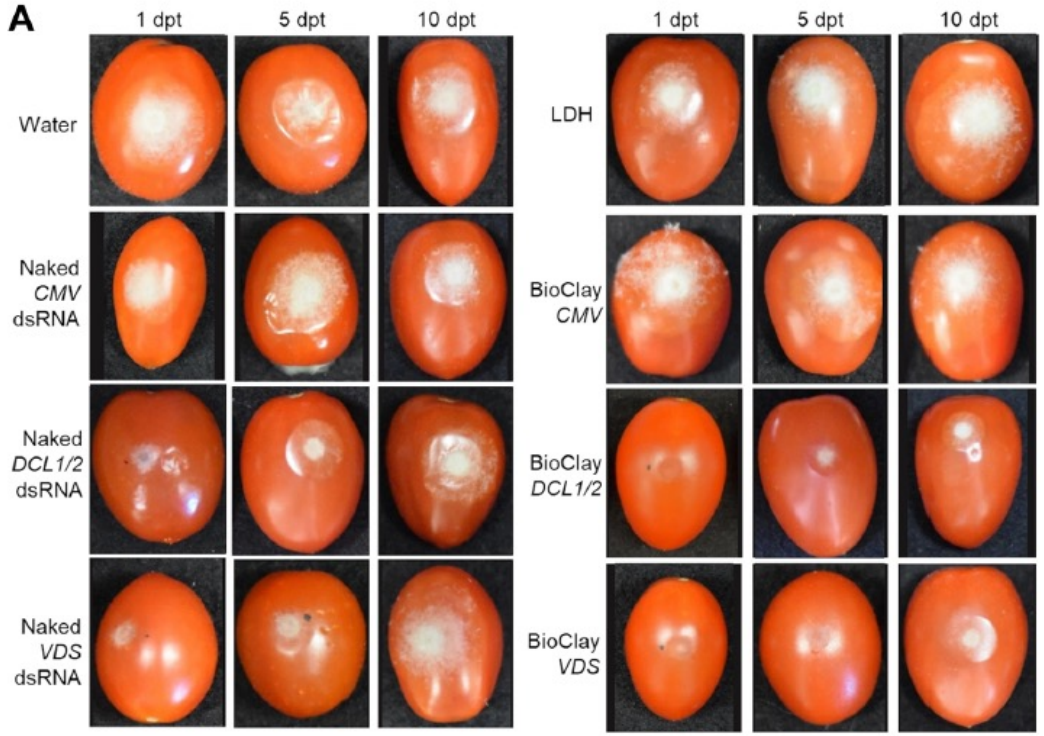
dsRNA treated

# Applications: Sprayable Biofungicide

## *Botrytis cinerea* protection

BioClay™ prolongs RNA interference-mediated crop protection against *Botrytis cinerea*

Niño-Sánchez *et al*. 2022. J *Integrat. Plant Biolo.* 11: 2187-2198.



# Applications: Beneficial Insect Health

## Protecting honeybees against Varroa mites

**Delivery pouch**

dsRNA is formulated into a sucrose solution and packaged into a pouch

Image courtesy of GreenLight Biosciences

# Applications: Beneficial Insect Health

## Silencing of secondary metabolites

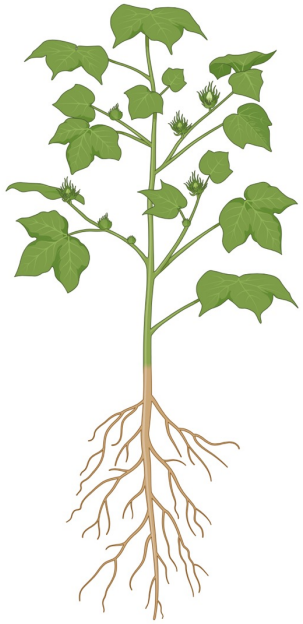
### Potato

- Glycoalkaloids

Paudel *et al.* 2017. *MPMI*, 30(11). 876-885.

### Cotton

- Gossypol



Rathore *et al*. 2020. *Crit Rev Plant Sci,* 39: 1-29.

# Regulatory Oversight for RNA-based Traits (PIPs) and Biopesticides

- **Environmental Protection Agency (EPA):** human health and environmental safety of dsRNA products
- **Food and Drug Administration (FDA):** safety of foods from crops containing or exposed to dsRNA
- **USDA:** risks to agriculture

# Regulatory Considerations for PIPs and Sprayable dsRNA

## Product composition and method of use factor into safety assessments

| | PIPs |
|---|---|
| **RNA molecule** | • Sequence specificity<br>• Degradation profile |
| **Method of manufacture** | • Expressed in the crop |
| **Formulation design** | • Plant matrix |
| **Application method** | • Expressed in crop |
| **Product use** | • Insect resistance trait |

# Regulatory Considerations for PIPs and Sprayable dsRNA

## Product composition and method of use factor into safety assessments

| | PIPs | Sprayable dsRNA |
|---|---|---|
| **RNA molecule** | • Sequence specificity<br>• Degradation profile | • Sequence specificity<br>• Degradation profile |
| **Method of manufacture** | • Expressed in the crop | • Enzymatic synthesis<br>• Microbial expression |
| **Formulation design** | • Plant matrix | • Liquids or dry formulations |
| **Application method** | • Expressed in crop | • Sprays |
| **Product use** | • Insect resistance trait | • Tank mix |

# Hazard Considerations for RNAi Products

## Human Health

Sequence-specific effects

- Bioinformatics analyses
- Tiered toxicity tests

Non-sequence specific effects

- Immune responses
- Toxicity of product formulation

## Environmental Safety

Sequence-specific effects

- Non-target organisms
- Bioinformatics analyses
- Tiered toxicity tests

# Exposure Considerations for RNAi Products

## Human Health

### Dietary exposure

- Both PIPs and spray
- Humans have barriers to ingested naked RNA

### Worker exposure

- Additional considerations for sprays based on product formulation and use
  - Inhalation
  - Dermal
  - Ocular

## Environmental Safety

### NTO exposure

- Potential for non-target organisms to be present in the crop
- Insect susceptibility to dsRNA

# Products: GM Virus Control

## Honeysweet plum: GM plum pox virus resistance

- Viral coat protein constructs resulted in gene silencing and resistance to virus
- EPA issued Section 3 registration on August 8, 2011

Scorza *et al.* 2013. *Plant Cell Tiss Organ Cult,* 115, 1–12

# Products: Genetically Modified Corn

SmartStax® PRO With RNAi TECHNOLOGY

## 3 modes of action

*DvSnf7* dsRNA + Cry3Bb1 + Cry34/35

- EPA Registration in June 2017
- Commercial release in 2022


BAYER Crop Science

Head *et al.* 2017. *Pest Mang. Sci.* 73: 1883-1899.
Darlington *et al.* 2022. *Insects.* 13, 57

# Products: Sprayable dsRNA

## Calantha™

dsRNA Spray-Induced Gene Silencing for Colorado Potato Beetle

EPA Registration
December 22, 2023

GreenLight™ Biosciences



Pallis *et al.* 2023. *Agriculture,* 13, 2283.
Pallis *et al*. 2023. *J Econ Entomol*, 116, 456-461.
Pallis *et al*. 2022. *Pest Manag Sci,* 78, 3836-3848.
Rodrigues *et al*. 2021. *Front Plant Sci,* 12, 728652.
Rodrigues *et al*. 2021. *American Chemical Society,* pp 65-82

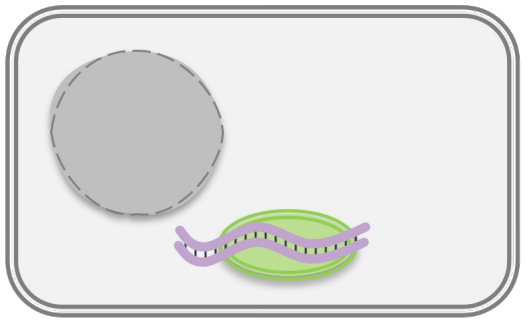
# Challenges

- **Technical challenges**
  - Recalcitrant pests and pathogens
  - RNA delivery
  - Resistance to dsRNA
  - Time and cost to develop
- **Regulatory timelines and harmonization across countries**
- **Public perception**

# Future Innovation in RNAi

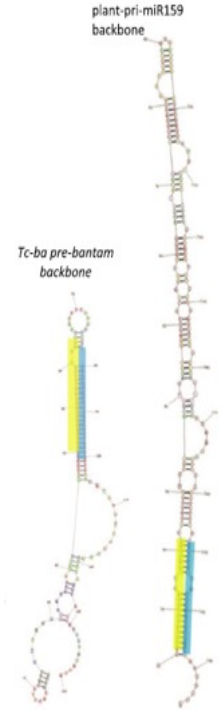
## New transgenic methods

### Chloroplast expression



Bally *et al.* 2008. *Pest Manag Sci*, 74, 1751-1758.

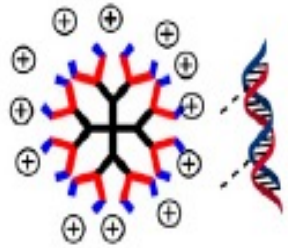
### Artificial miRNA



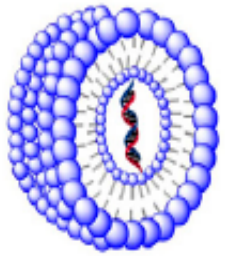

Bally *et al.* 2020. *Plant Biotechnol J,* 18, 1925-1932

## New sprayable formats

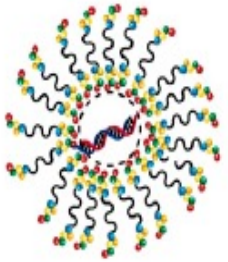
### RNA carriers



Polymers



Lipids

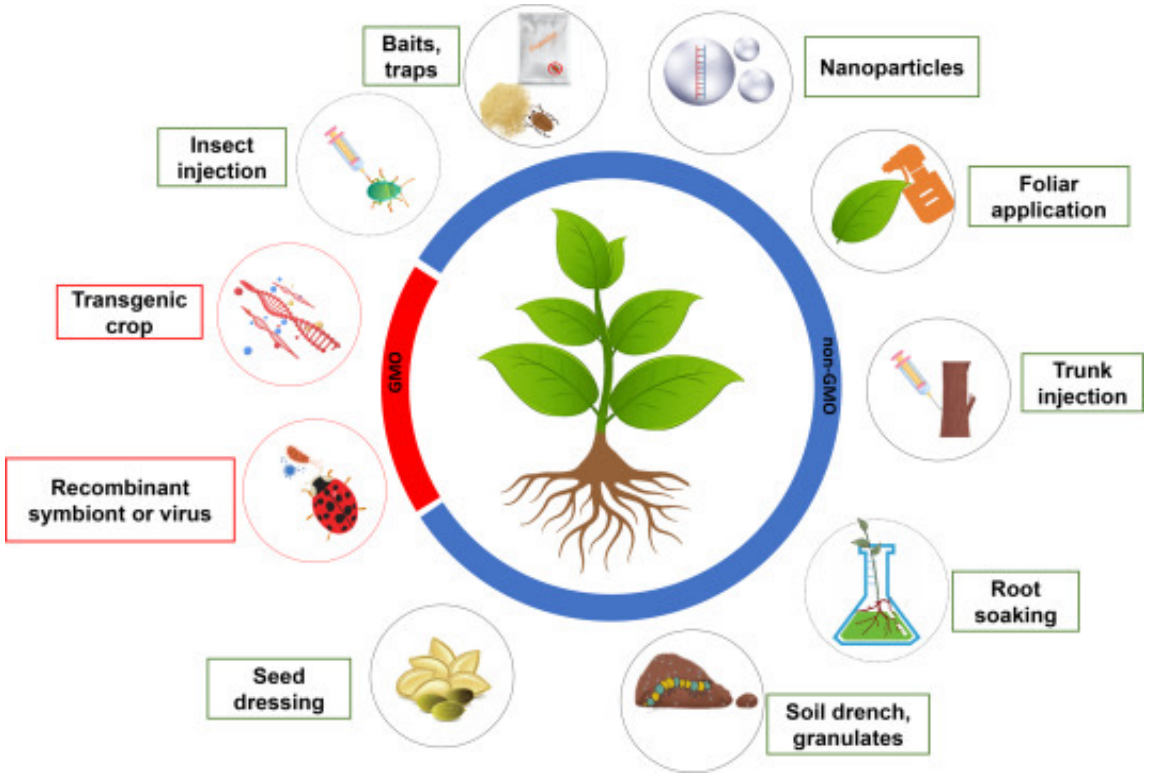


Peptides

Yang *et al.* 2022. *Front. Bioeng. Biotechnol.*, 10:974646.

# Future Innovation in RNAi

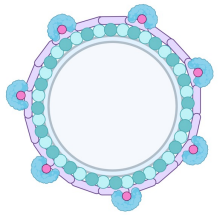
## New applications

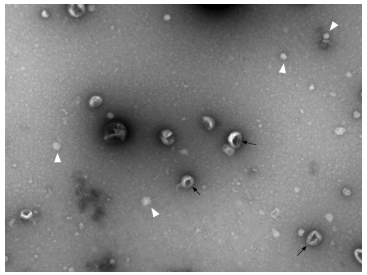



Liu *et al*. 2020. *Biotechnology Advances, 39: 107463.*

## New research

### Extracellular vesicles





### Non-canonical RNAi mechanisms

#### piRNAi Pathway

Mondal *et al. 2020. Life Science Alliance.* e202000731

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## Questions?

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## Developing and Adopting Economically Effective Mitigation Strategies: Critical to the Survival of Agriculture and Endangered Species

Tuesday, Jan. 30, from 12-1 p.m. CST.

Register Now

### Upcoming Webinars

| | |
|---|---|
| **January 30** | Developing and Adopting Economically Effective Mitigation Strategies: Critical to the Survival of Agriculture and Endangered Species |
| **February 20** | FIFRA, ESA and Pesticide Consultation: Understanding and Addressing the Complexities |
| **March 12** | The topic of this webinar is the role of states in the implementation and regulation of FIFRA. Exact content will be announced later. |

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